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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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YOUNG & THOMPSON
745 SOUTH 23RD STREET
2ND FLOOR
ARLINGTON, VA 22202

EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 07/26/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/764,581	SAKANYAN ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 May 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-16* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *27 January 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *4/21/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The Art Unit location of your application in the USPTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Art Unit 1636.

Receipt is acknowledged of an amendment, filed 5/9/2005, in which claims 17-27 were canceled.

Election/Restrictions

Applicant's election without traverse of Group I (claims 1-16) in the reply filed on 5/9/2005 is acknowledged. An examination on the merits of claims 1-16 follows.

Information Disclosure Statement

Receipt of an information disclosure statement, filed on 4/21/2004, is acknowledged. The signed and initialed PTO 1449 has been mailed with this action.

Priority

Acknowledgment is made of applicant's claim for foreign priority under 35 U.S.C. 119(a)-(d). Receipt of the certified copy of the foreign priority document, EPO 01402049.9, is acknowledged. These papers have been placed of record in the file.

Specification

The disclosure is objected to because of the following informalities: numerous letters are missing from the words at the top and/or bottom of pages 4-8, 15-18, 33, 41, 42, 44, 49, 50 and

53. Applicant should review all pages of the specification for missing letters and correct all occurrences. Appropriate correction is required.

The disclosure is objected to because of the following informalities: at pages 27-28, Table 1 appears to be missing text along the left side of the table. Appropriate correction is required.

Claim Objections

Claim 1 is objected to because of the following informalities: the word "and" should be placed between steps (a) and (b) to improve the grammar of the claim. Appropriate correction is required.

Claim 8 is objected to because of the following informalities: the word "extracts" in line 2 of the claim should be changed to "extract" to improve the grammar of the claim. Appropriate correction is required.

Claim 9 is objected to because of the following informalities: the word "and" should be placed between steps (c) and (d) to improve the grammar of the claim. Appropriate correction is required.

Claim 14 is objected to because of the following informalities: the word "of" should be placed after the word "downstream" to improve the grammar of the claim. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-8, 11 and 14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is vague and indefinite in that the metes and bounds of the phrase "enabling RNA or polypeptide synthesis from a DNA template" are unclear. The phrase is unclear in that the dependent claims further limit the structure of the DNA. However, the method does not comprise a step of adding the DNA to the cell-free system. Thus, it is unclear if the method encompasses the step of adding the DNA template to the cell-free system or is only capable of transcribing the claimed DNA template.

Claim 1 is vague and indefinite in that the metes and bounds of the phrase "comparing to its natural concentration existing in the cell-free system" are unclear. The phrase is unclear in that a cell-free system may be made from a cell extract, which would have a "natural concentration" of RNA polymerase, or a cell-free system can be assembled from synthetic materials. In a synthetic system, the concentration of the RNA polymerase would depend only on the amount added to the reaction and would have no "natural concentration". Thus, the metes and bounds of the "natural concentration" are unclear. It would be remedial to amend the claim language to clearly indicate how the concentration of the alpha subunit is compared to the other subunits of the RNA polymerase.

Regarding claim 3, the phrase "preferably" renders the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

Claims 7 and 11 are vague and indefinite in that the metes and bounds of the phrase "comprised between 15 μg/ml and 200 μg/ml" are unclear. The phrase is unclear in that the term "comprised" is inclusive or open-ended and does not exclude additional, unrecited elements such as additional purified alpha subunit. However, the claim also recites a narrower range of 15 μg/ml and 200 μg/ml. A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). For the purposes of examination, the range is interpreted as encompassing less than 15 μg/ml, 15 μg/ml to 200 μg/ml, and more than 200 μg/ml.

Claim 8 is vague and indefinite in that the metes and bounds of the phrase "wherein the cell-free extracts is prepared from cells overexpressing a gene encoding α subunit of RNA polymerase" are unclear. The phrase appears to modify the cell-free extract to increase the amount of RNA polymerase alpha subunit. However, claim 6 requires that the concentration of the RNA polymerase be increased by the addition of "purified α subunit of RNA polymerase to the bacterial cell-free extract." The cell-free extract obtained from a cell overexpressing a gene encoding an alpha subunit of RNA polymerase does not constitute a purified alpha subunit. For the purposes of examination, the phrase has been interpreted as describing the source of the purified alpha subunit of RNA polymerase.

Regarding claim 14, the phrases "preferably" and "more preferably" render the claim indefinite because it is unclear whether the limitation(s) following the phrases are part of the claimed invention. See MPEP § 2173.05(d).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 1 is rejected under 35 U.S.C. 102(b) as being anticipated by Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) as evidenced by Inouye et al (Nucleic Acids Research, Vol. 13, No. 9, pages 3101-3110, 1985; see the entire reference).

Bowrin et al teach a method for *in vitro* transcription, comprising the steps of (i) providing a cell-free transcription system, wherein the concentration of the alpha subunit of RNA polymerase is increased relative to the other subunits of the RNA polymerase, and (ii) recovering the synthesized RNA transcript (e.g. pages 2-3, *In vitro transcription assays*; pages 4-5, *Effect of α subunit on cell-free ompF transcription*; Figure 5). Bowrin et al teach that the cell-free system is capable of enabling RNA synthesis form a DNA template comprising an *lpp* promoter (e.g. Figure 5). The teachings of Bowrin et al read on the claimed invention because the *lpp* promoter inherently contains an UP element (Inouye et al, e.g. Figure 1). As disclosed in the instant specification, the UP element is an AT-rich sequence located upstream at the –35 site of strong promoters (e.g. page 3, lines 15-20). The –35 region of the *lpp* promoter contains an AT-rich region (Inouye et al, e.g. Figure 1). Thus, the cell-free system of Bowrin et al enables transcription from a DNA template comprising a promoter with at least one UP element.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 2, 6, 7 and 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lesley et al (The Journal of Biological Chemistry, Vol. 266, No. 4, pages 2632-2638, 1991; see the entire reference) in view of Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) as evidenced by Bowrin, Brissette and Inouye (Journal of Bacteriology, Vol. 174, No. 20, pages 6685-6687, 1992; see the entire reference).

Regarding claims 1, 2, 6 and 7, Lesley et al teach a method for coupled *in vitro* transcription-translation, comprising (i) providing a bacterial, S-30 cell-free system for *in vitro* transcription-translation, and (ii) recovering the synthesized polypeptide (e.g. page 2633, *S-30 Preparation and Reactions and Core-binding Assays*). Lesley et al teach that a variety of DNA

templates may be used, including supercoiled plasmid, restriction fragments, and PCR-generated templates (e.g. page 2634, paragraph bridging columns). Further, Lesley et al teach the addition of isolated core RNA polymerase to the reaction (e.g. page 2633, paragraph bridging columns). Absent any evidence to the contrary, the bacterial cell-free system of Lesley et al would allow transcription of DNA templates comprising a bacterial promoter with at least one UP element. As disclosed in the instant specification, the UP element is bound by the RNA polymerase alpha subunit (e.g. paragraph bridging pages 3-4), which is present in the S-30 extract of Lesley et al.

Regarding claims 9-14, Lesley et al teach a method for coupled *in vitro* transcription-translation, comprising (i) providing an S-30 cell-free system for *in vitro* transcription-translation, (ii) adding the DNA template, and (iii) recovering the synthesized polypeptide (e.g. page 2633, *S-30 Preparation and Reactions and Core-binding Assays*). Lesley et al teach that a variety of DNA templates may be used, including supercoiled plasmid, restriction fragments, and PCR-generated templates (e.g. page 2634, paragraph bridging columns). Regarding PCR generated templates, Lesley et al teach the amplification of an open reading frame, wherein the DNA fragment produced comprises 3 bp of additional sequence immediately downstream of the first stop codon (e.g. page 2633, *PCR Methods*; page 2634, Results; Table I). Further, Lesley et al teach the addition of isolated core RNA polymerase to the reaction (e.g. page 2633, paragraph bridging columns). Lesley et al teach the absence of the optionally claimed step of "adding a thermostable polymerase." Further, Lesley et al teach the use of the cell-free system to transcribe a DNA template comprising an amplification product of an open reading frame of the sigma protein (e.g. paragraph bridging pages 2632-2633; page 2633, *PCR Methods*; Table 1; page 2634, paragraph bridging columns). Absent any evidence to the contrary, the cell-free

system of Lesley et al would allow transcription of DNA templates comprising a promoter with at least one UP element. As disclosed in the instant specification, the UP element is bound by the RNA polymerase alpha subunit (e.g. paragraph bridging pages 3-4), which is present in the S-30 extract taught by Lesley et al.

Furthermore, Lesley et al teach the use of the cell-free system to express a protein from a DNA template and test for the ability of the produced protein to interact with exogenously supplied isolated core RNA polymerase (e.g. pages 2635-2636, *Assay of Core Binding Activity of Protein Fragments*; Figure 4). Lesley et al exemplify the binding assay with sigma protein (e.g. Figure 4). However, Lesley et al teach that the method has other applications with other types of proteins such as mapping epitopes of monoclonal antibodies (e.g. page 2633, left column, 1st full paragraph).

Lelsey et al do not teach the addition of the alpha subunit of RNA polymerase or a DNA-binding regulatory protein to the cell-free system.

Bowrin et al teach a method for *in vitro* transcription, comprising the steps of (i) providing a cell-free transcription system, wherein the concentration of the alpha subunit of RNA polymerase is increased relative to the other subunits of the RNA polymerase, and (ii) recovering the synthesized RNA transcript (e.g. pages 2-3, *In vitro transcription assays*; pages 4-5, *Effect of α subunit on cell-free ompF transcription*; Figure 5). Bowrin et al teach the *in vitro* transcription of plasmid pKI0033, containing the *OmpF* promoter, in a reaction comprising purified RNA polymerase alpha subunit and OmpR protein (e.g. pages 2-3, *In vitro transcription assays*; Figure 4). Further, Bowrin et al teach the *in vitro* transcription of plasmid pKEN022, containing the *lpp* promoter (e.g. Figure 5). This promoter is used as a control to demonstrate that the inhibition of

transcription by the alpha subunit of RNA polymerase is specific to the *OmpF* promoter (e.g. page 5, paragraph bridging columns). Bowrin et al teach the addition of 25-100 ng of purified alpha subunit to the reaction taught by Bowrin, Brissette and Inouye. Bowrin, Brissette and Inouye teach a reaction volume of 19.5 µl (e.g. paragraph bridging pages 6685-6686). Thus, Bowrin et al teach the addition of purified RNA polymerase alpha subunit at a concentration of 1.3 µg/ml to 5.1 µg/ml. Bowrin et al state that "It has been suggested by several groups...that α is the subunit of RNA polymerase that interacts with Class I transcription factors, a group to which OmpR belongs" (see page 5, Conclusion).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method and analysis of protein interactions of Lesley et al to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al because Lesley et al and Bowrin et al teach it is within the ordinary skill in the art to use in vitro transcription reactions to study protein interaction and transcription.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to test the effect of RNA polymerase alpha subunit and OmpR protein on OmpF transcription as taught by Bowrin et al and of being able to test the binding of OmpR and RNA polymerase alpha subunit in the same reaction as taught by Lesley et al and suggested by Bowrin et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1-3, 6, 7 and 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lesley et al (The Journal of Biological Chemistry, Vol. 266, No. 4, pages 2632-2638, 1991; see the entire reference) in view of Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) and Savchenko et al (Gene, Vol. 212, pages 167-177, 1998; see the entire reference) as evidenced by Bowrin, Brissette and Inouye (Journal of Bacteriology, Vol. 174, No. 20, pages 6685-6687, 1992; see the entire reference).

The teachings of Lesley et al are described above and applied as before.

Lelsey et al do not teach the addition of the alpha subunit of RNA polymerase or a DNA-binding regulatory protein to the cell-free system. Further, Lesley et al do not teach the *in vitro* transcription of a DNA template further comprising a sequence from the argC promoter of *Bacillus stearothermophilus*.

The teachings of Bowrin et al are described above and applied as before.

Savchenko et al teach multiple plasmid vectors comprising the argC promoter (i.e. Parg) (e.g. Table 1; Figures 1 and 2). Savchenko et al teach that pHAV2, comprising an argC promoter, always directed higher expression in *E. coli* as compared to pBTargC, which comprises a Ptac promoter (e.g. page 173, section 3.2). The Ptac promoter is considered to be a powerful promoter (e.g. page 173, section 3.2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method and analysis of protein interactions of Lesley et al to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al because Lesley et al and Bowrin et al teach it is within the ordinary skill in the art to use in vitro transcription reactions to

study protein interaction and transcription. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the control plasmid comprising the *lpp* promoter with a plasmid comprising an argC promoter because Lesley et al teach a bacterial expression system and Savchenko et al teach the expression from the argC promoter in *E. coli*.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to test the effect of RNA polymerase alpha subunit and OmpR protein on OmpF transcription as taught by Bowrin et al and of being able to test the binding of OmpR and RNA polymerase alpha subunit in the same reaction as taught by Lesley et al and suggested by Bowrin et al. Further, one would have been motivated to replace the control plasmid with a plasmid comprising an argC promoter because Savchenko et al teach that the argC is a strong promoter relative to other strong promoters known in the art and because it would be easier to detect a transcript expressed from a stronger promoter. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 2, 6, 7-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lesley et al (The Journal of Biological Chemistry, Vol. 266, No. 4, pages 2632-2638, 1991; see the entire reference) in view of Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) and Fujita et al (Methods in Enzymology, Vol. 273, pages 121-

130, 1996; see the entire reference) as evidenced by Bowrin, Brissette and Inouye (Journal of Bacteriology, Vol. 174, No. 20, pages 6685-6687, 1992; see the entire reference).

The teachings of Lesley et al are described above and applied as before.

Lelsey et al do not teach the addition of the alpha subunit of RNA polymerase or a DNA-binding regulatory protein to the cell-free system. Further, Lesley et al do not teach the step of increasing the concentration of the alpha subunit of RNA polymerase by adding a purified alpha subunit isolated from cells overexpressing a gene encoding an alpha subunit of RNA polymerase.

The teachings of Bowrin et al are described above and applied as before.

Fujita et al teach *E. coli* transformed with the expression plasmid pGEMAX185, which expresses the RNA polymerase alpha gene upon the addition of IPTG (e.g. pages 122-123, Wiled-type α subunit). Fujita et al teach that the level of plasmid-encoded alpha subunit is more than 100-fold higher than that of chromosome-encoded alpha (e.g. pages 122-123, Wiled-type α subunit). Fujita et al teach the isolation of RNA polymerase alpha subunit with a yield of about 5 mg and a purity of more than 95% (e.g. pages 122-123, Wiled-type α subunit).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method and analysis of protein interactions of Lesley et al to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al because Lesley et al and Bowrin et al teach it is within the ordinary skill in the art to use in vitro transcription reactions to study protein interaction and transcription. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to purify the alpha subunit of the

RNA polymerase because Fujita et al tech a method of isolation and Bowrin et al teach the use of isolated RNA polymerase alpha subunit.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to test the effect of RNA polymerase alpha subunit and OmpR protein on OmpF transcription as taught by Bowrin et al and of being able to test the binding of OmpR and RNA polymerase alpha subunit in the same reaction as taught by Lesley et al and suggested by Bowrin et al. Further, one would have been motivated to use the method of Fujita et al to obtain the purified alpha subunit of RNA polymerase because Fujita et al teach a yield great enough for multiple reactions based upon the amount of protein taught by Bowrin et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 2, 4-7 and 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lesley et al (The Journal of Biological Chemistry, Vol. 266, No. 4, pages 2632-2638, 1991; see the entire reference) in view of Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) and Xue et al (Biochemistry, Vol. 39, pages 14356-14362, 2000; see the entire reference) as evidenced by Bowrin, Brissette and Inouye (Journal of Bacteriology, Vol. 174, No. 20, pages 6685-6687, 1992; see the entire reference).

The teachings of Lesley et al are described above and applied as before.

Lelsey et al do not teach the addition of the alpha subunit of RNA polymerase or a DNA-binding regulatory protein to the cell-free system. Further, Lesley et al do not teach the addition

of a thermostable RNA polymerase enzyme or *Thermus thermophilus* RNA polymerase to the cell-free system.

The teachings of Bowrin et al are described above and applied as before.

Xue et al teach the isolation of the DNA-dependent RNA polymerase (RNAP) from the thermophile *Thermus thermophilus* (e.g. pages 14356-14357, Materials and Methods). Xue et al teach an *in vitro* transcription reaction comprising the *T. thermophilus* RNAP and a DNA template obtained as a PCR fragment (e.g. page 14358, right column, 1st full paragraph). Xue et al teach that the *T. thermophilus* RNAP is capable of initiating and elongating transcription at higher temperatures than *E. coli* RNAP (e.g. paragraph bridging pages 14360-14361; Figures 8 and 9).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method and analysis of protein interactions of Lesley et al to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al because Lesley et al and Bowrin et al teach it is within the ordinary skill in the art to use in vitro transcription reactions to study protein interaction and transcription. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method of Lesley et al to include the addition of the purified RNAP of *Thermus thermophilus* because Xue et al teach that the *T. thermophilus* RNAP is capable of functioning in *in vitro* transcription reactions using a DNA template that is capable of being transcribed by *E. coli* RNAP.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to test the effect of RNA polymerase alpha subunit and OmpR protein on OmpF transcription as taught by Bowrin et al and of being able to test the binding of OmpR and RNA polymerase alpha subunit in the same reaction as taught by Lesley et al and suggested by Bowrin et al. Further, one would have been motivated to modify the cell-free system to include the *T. thermophilus* RNAP to increase the amount of transcription based upon the increased amount of polymerase present and to increase the range of temperature at which the cell-free system will be capable of transcribing DNA template into RNA. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1, 2, 6, 7 and 9-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lesley et al (The Journal of Biological Chemistry, Vol. 266, No. 4, pages 2632-2638, 1991; see the entire reference) in view of Bowrin et al (FEMS Microbiology Letters, Vol. 115, pages 1-6, 1994; see the entire reference) and Uptain et al (PNAS, Vol. 94, pages 13548-13553, 1997; see the entire reference) as evidenced by Bowrin, Brissette and Inouye (Journal of Bacteriology, Vol. 174, No. 20, pages 6685-6687, 1992; see the entire reference).

The teachings of Lesley et al are described above and applied as before.

Lelsey et al do not teach the addition of the alpha subunit of RNA polymerase or a DNA-binding regulatory protein to the cell-free system. Further, Lesley et al do not teach the *in vitro*

transcription of a DNA template further comprising a transcriptional terminator, or the T7 phage transcriptional terminator.

The teachings of Bowrin et al are described above and applied as before.

Uptain et al teach that transcriptional terminator sequences, such as the phage T7 early site terminator, can be added to the 3' end of a transcribed region for *in vitro* transcription (e.g. page 13549, DNA Templates, Semi-Solid State in Vitro Transcription Reactions; Table 1). Uptain et al teach that *E. coli* RNA polymerase is capable of intrinsic and rho-dependent termination on the T7 early terminator (e.g. page 13552, left column). Further, Uptain et al teach that terminators cause the RNA polymerase to release the transcript.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the cell-free transcription-translation method and analysis of protein interactions of Lesley et al to include the addition of plasmid pKI0033 and purified RNA polymerase alpha subunit and OmpR protein as taught by Bowrin et al because Lesley et al and Bowrin et al teach it is within the ordinary skill in the art to use in vitro transcription reactions to study protein interaction and transcription. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the DNA template of Lesley et al to include a T7 transcriptional terminator because Lesley et al teach it is within the skill of the art to use different types of DNA templates and Uptain et al teach the inclusion of the T7 terminator in a template for *in vitro* transcription.

One would have been motivated to make such a modification in order to receive the expected benefit of being able to test the effect of RNA polymerase alpha subunit and OmpR protein on OmpF transcription as taught by Bowrin et al and of being able to test the binding of

OmpR and RNA polymerase alpha subunit in the same reaction as taught by Lesley et al and suggested by Bowrin et al. Further, one would have been motivated to include the T7 terminator of Uptain et al in order to receive the expected benefit of more efficiently terminating transcription to obtain transcripts of more uniform length and leave the RNA polymerase free to re-initiate transcription, resulting in increased yield. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached at 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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Jennifer Dunston
Examiner
Art Unit 1636

jad

TERRY MCKELVEY
PRIMARY EXAMINER